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PESTICINOGENY: AN IDENTIFYING CHARACTERISTIC OF PASTEURELLA PESTIS

Earl D. Beesley
Michael J. Surgalla

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DEPARTMENT OF THE ARMY
Fort Detrick
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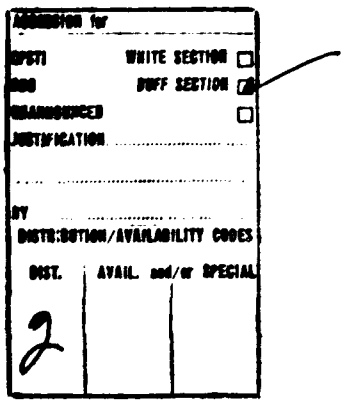

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PESTICINOGENY: AN IDENTIFYING CHARACTERISTIC OF *PASTEURELLA PESTIS*

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MEDICAL SCIENCES LABORATORIES

Project 1B562602A059

January 1969

ABSTRACT

Current methods of identifying Pasteurella pestis rely heavily on tests specific for detecting fraction I, the envelope antigen. Pesticin I, a bacteriocin inhibitory for Pasteurella pseudotuberculosis, has been demonstrated in nearly all strains isolated from human infections. The results of use of this characteristic as an identifying trait for P. pestis were compared with results reported for detecting fraction I by fluorescent antibody and antiserum agar techniques. Data indicate that although certain atypical strains of P. pestis fail to react in one system or the other, a combination of these tests provides positive identification in all cases. The rapidity, simplicity, and specificity of this test makes it a valuable tool in the study of plague infections and an important adjunct to methods currently in use.

I. INTRODUCTION*

The identification of Pasteurella pestis, until recently, has been a time-consuming problem. Confirmation of the identity of the infecting organisms by accepted morphological, biochemical, antigenic, and animal infectivity studies requires approximately 2 weeks. However, with the development[1] of fluorescent antibody specific for Fraction I, the envelope antigen of P. pestis, it has become possible to provide a presumptive diagnosis of plague within a matter of hours. Since many hospitals are not equipped to perform fluorescent antibody examination of specimens for the presence of P. pestis, it seems important that alternative procedures should be available to provide the clinical microbiologist a tool for rapid plague detection. One such highly specific method has been described[2] that uses a nutrient medium containing antiserum specific for Fraction I. This report discusses the application of the pesticin assay[3] for detection of pesticin I in rapid presumptive identification of P. pestis.

II. MATERIALS AND METHODS

Pesticin I is detected by its ability to inhibit growth of type I Pasteurella pseudotuberculosis, a species closely related to P. pestis. The major requirements for a successful assay are: (i) a minimum level of Fe^{++} and (ii) an excess of free Ca^{++}. These conditions are satisfied by including calcium ethylenediaminetetraacetic acid (Ca-EDTA) in the medium. A proven formula for pesticin I assay agar, essentially the same as described by Brubaker and Surgalla[4] consists of 4% blood agar base** in distilled water. After sterilization at 121 C for 15 min, the agar is cooled to 45 to 50 C and sterile $CaCl_2$, Ca-EDTA, and glucose are added to give final concentrations of 0.01M, 0.1%, and 0.01M respectively. The base layer is poured, solidified, and allowed to dry at room temperature.

Specimens may then be streaked to insure that developing colonies are well separated. Isolated colonies appear after 24 hours at 26 C and reach a diameter of approximately 1 to 1.5 mm in 48 hours.

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** Baltimore Biological Laboratories.

Each plate is inverted for 1 min over a 2 in^2 gauze pad saturated with chloroform. This sterilizes the surface of the colonies and prevents the cells from mixing with the indicator layer. After excess chloroform is allowed to dissipate, melted pesticin agar is cooled to 45 C and inoculated with type I P. pseudotuberculosis so that each ml contains 1 x 10^5 organisms. Five ml of this suspension is then pipetted evenly over the surface of the suspected colonies. Plates incubated at 37 C may be examined after 18 to 24 hours. Wide, clear zones of inhibition appear around colonies producing pesticin I (Fig. 1).

Moderately contaminated samples may be streaked directly on pesticin assay medium. A more satisfactory method is to dilute the specimen and inoculate the agar with several levels to insure the best possible separation of colonies.

The results of a typical test are shown in Figure 2. The sample, bone marrow from an exhumed body, yielded 1.5 x 10^6 P. pestis per gram of tissue. This was roughly 1% of the total viable organisms recovered.

When pure cultures are available, one plate may be used conveniently to test as many as six unknowns (Fig. 3).

It should be strongly emphasized, however, that positive and negative controls must be included with each test to insure that the system is functioning properly.

FIGURE 1. Clear Zones Around Isolated Colonies of Pasteurella pestis Demonstrate Pesticin I Inhibition of Pasteurella pseudotuberculosis. Note positive control in center.

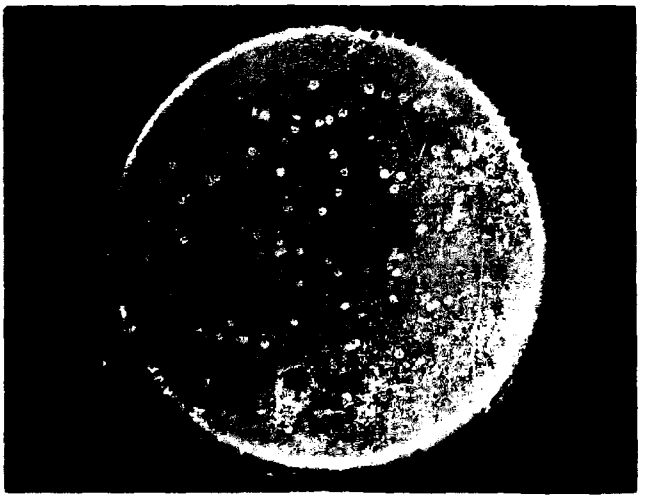

FIGURE 2. Demonstration of the Pesticin I Assay for Detecting Pasteurella pestis in Contaminated Specimens.

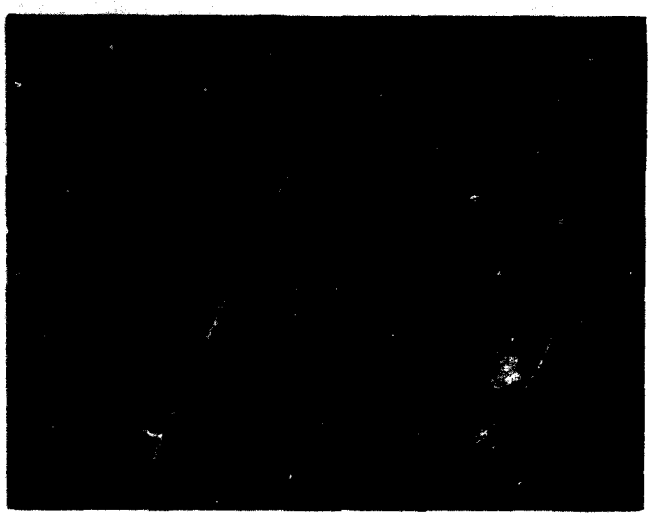

FIGURE 3. Pesticin I Assay Used for Testing Several Pure Cultures at One Time. Note positive control in center and negative control at top.

III. RESULTS AND DISCUSSION

Pesticin, a bacteriocin-like substance produced by P. pestis, was first described by Ben-Gurion and Hertman.[5] Studies by Brubaker and Surgalla[6] revealed the presence of two bacteriocins, pesticin I and pesticin II. Correlation of pesticin I production with the expression of fibrinolytic activity and coagulase was reported by Brubaker et al.[8] This relationship was further studied by Beesley et al.[7]

The loss of pesticinogeny and the genetically linked fibrinolytic factor and coagulase also reduce the organisms' invasive powers.[8]

This type of organism is apparently quite rare in natural plague infections. The literature covers only two strains isolated from nature that lack this property and yet retain other known virulence factors. One of these was isolated from a flea and is not pertinent here. The other was recovered from a human infection. Strain Dodson, reported by Surgalla and Beesley,* was isolated from a young boy in Arizona in 1967. The apparent rarity of this type of organism in human and sylvatic plague indicates the high stability of pesticinogeny and supports its value in identifying P. pestis.

The wide variation in the genetic make-up of P. pestis[9] points out the need for new methodology that can be applied at the level of the hospital diagnostic laboratory. Table 1 shows some of the known varieties of the plague organism and indicates two properties that differentiate between P. pestis and P. pseudotuberculosis. Table 2 shows how a combination of testing procedures can decrease the chance of misdiagnosing plague.

TABLE 1. EXPRESSION OF BACTERIAL PROPERTIES BY P. PESTIS AND P. PSEUDOTUBERCULOSIS

Strain	Fraction I	Pesticin I
	P. pestis	
Alexander	+	+
Dodson	+	-
Bryans	+	+
M23	-	+
	P. pseudotuberculosis	
Alaska	-	-
PB1/+	-	-

* Unpublished data, 1968.

TABLE 2. METHODS FOR IDENTIFYING P. PESTIS

Strain	Fluorescent Antibody	Antiserum Agar	Pesticin I Assay	Phage	Guinea Pig Lethality
		P. pestis			
Alexander	+	+	+	+	+
Dodson	+	+	0	+	±
Bryans	0	+	+	+	±
M23	0	0	+	+	±
		P. pseudotuberculosis			
Alaska	+	0	0	0	0
PB1/+	0	0	0	0	0

± = Inconsistent.

The use of the pesticin I assay has several important advantages, especially in diagnosis of human infections. First, the method is easy to use, and extensive training is unnecessary. Secondly, there is no requirement for expensive and unusual equipment. The basic materials used in the assay are inexpensive, quite stable, and easily obtained. Suitable avirulent strains of *P. pseudotuberculosis* also are readily available. Third, unlike testing for phage sensitivity, pure cultures are not necessary if the colonies are sufficiently separated on the initial isolation plate.

We do re-emphasize that the use of the pesticin I assay is not intended to replace other accepted techniques, but rather to supplement them and increase the effectiveness of plague investigation.

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14. Key Words

*Pasteurella pestis
*Pesticins
*Identification
Pasteurella pseudotuberculosis
Detection
Antigens
Bacteriocins
Inhibitors
Fluorescent antibody
Antiserums
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